

CHEMICAL TRANSMISSION OF THE EFFECTS OF NERVE IMPULSES*

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INTRODUCTORY

The term "humoral transmission" was used by Otto Loewi (1921) in describing the first direct demonstration of the process which forms the subject of my lecture. There appears to have been some uncertainty as to whether the term "humoral" referred to the experimental transfer on which that demonstration was based, or to the natural process of release of a specific stimulant into the tissue fluids. In any case I shall have to consider some instances in which the chemical transmitter of nervous effects appears to be released in such immediate proximity to the receptive cells that the use of the term "humoral" would risk a misleading implication. For this reason I have chosen to employ the more general term "chemical transmission" for the process, and shall refer to the agents concerned as "chemical transmitters."

PHYSIOLOGY OF TRANSMISSION

The transmission of the effects of impulses in nerve fibres, to awaken or to modify the activity of cells in relation to which the nerve fibres end, is one of the classical problems of physiology; and the classical subject for its experimental study has been the familiar preparation of motor nerve and voluntary muscle.

Since the experiments of Claude Bernard it has been known that the point where the nerve fibre ends, on the end-plate of the muscle fibre, has special physiological properties. If the response of the muscle to a nerve impulse is paralysed by curare or by fatigue, it is here that the excitatory process is blocked, while nerve fibre and muscle fibre are still normally responsive, and still normally conduct. The fact that the transmission of excitation is peculiarly liable to interruption at this point would not by itself imply that a different process or mechanism of transmission here intervened. It might merely indicate that structures using the same process of conduction as nerve and muscle were here most readily accessible to certain poisons or to the depressant effect of fatigue. Lapique explains it as due to a change in the chronaxie of the muscle fibres. And I think that I am right in supposing that the prevalent conception of the excitation of a voluntary muscle fibre by a nervous impulse assumes that the wave of physio-chemical disturbance, propagated along the nerve fibre as the nervous impulse, passes directly to the muscle fibre, and there excites contraction as it is further propagated.

This conception of the unbroken physical transmission of the excitation wave from nerve to muscle might well seem to receive support from the analogy between the nerve-muscle junction and a synapse of the nervous system. In both cases we have the terminal branching of a nerve fibre, the axon process of a neurone, making contact with another cell—the cell body of another neurone or a muscle fibre. In the case of the synapse the response excited is a nerve impulse in the axon of the second neurone, essentially similar to that which is conducted to the synapse by the axon of the first. The suggestion of unbroken propagation is strong; and if such continuity of conduction occurs at a synapse there is no obvious reason why it should not occur at a nerve-muscle junction.

There can be no suggestion, however, that all the

events at a synapse can be described in terms of the simple conduction of an impulse. Whereas conduction is equal in either direction in nerve or muscle fibre, the excitation can pass a synapse or a nerve-muscle junction in only one direction. Sherrington and his pupils, in their great analysis of central reflex action, have considered such phenomena as recruitment, the subliminal excitatory state, after-discharge, and inhibition; and Sherrington (1925) has been led thereby to a clear recognition of something, whether a chemical substance or a state, persisting at a synapse well beyond the duration of the incident impulse. Further, with regard to the transmission of excitation from motor nerve to voluntary muscle, Adrian (1933), in a recent article, admits that it may not be so fundamentally different from that which we shall presently consider in the case of autonomic nerves, but that "an excitatory substance liberated at a nerve ending, but destroyed within a few thousandths of a second . . . would account well enough for the known properties of a nerve ending."

The direct evidence, however, for the intervention of such a chemical transmitter between nerve impulse and effector cell came, in the first instance, from studies of the nervous control of the activities of involuntary muscle and gland cells by nerves of the autonomic system. Since it is the theme of my lecture, I think it will be proper to attempt to trace the conception to its origin, and to pass in brief review the stages in the unfolding of the story. Naturally I cannot, in a lecture, attempt a comprehensive and detailed record of the evidence to which many have contributed, and I must select for mention, not necessarily those items which are more important than others, but those which seem to suit my purpose of telling a coherent story.

EARLY SUGGESTIONS OF CHEMICAL TRANSMISSION

The suggestion that nervous effects might be transmitted by the release of a specific chemical stimulant was first made in 1904 by T. R. Elliott, then working as George Henry Lewes student in the department of physiology at Cambridge. He had just worked out in detail the now familiar correspondence between the actions of adrenaline and those of true sympathetic nerves; and I myself had the pleasure of co-operating with him in a few experiments which showed that this correspondence extended to the selective action of ergotoxine, which paralysed the same group of effects in the actions of adrenaline and of sympathetic nerves.

Elliott advanced, in explanation, the daring idea that sympathetic nerve fibres liberate adrenaline at their endings, to act as the transmitter and immediate agent of their effects. The years have justified his courageous insight, but I think that the late W. E. Dixon was almost alone at that time in seizing the idea with eager conviction. Dixon (1906, 1907) went further, to argue that parasympathetic nerves must similarly release a chemical transmitter of their effects. There was nothing then known in the body to play this part, and Dixon could only think of the parasympathetic transmitter as muscarine. He did, however, make an experimental attempt to find evidence of its release in the mammalian heart when the vagus nerves were stimulated. Removing a dog's heart while it was under vagus inhibition, he made, concentrated, and partially purified an extract from it; and he found that

* The Linacre Lecture of St. John's College, Cambridge, delivered on May 5th, 1934, in the Department of Physiology, Cambridge.

this, when applied to the beating frog's heart, had an inhibitor effect which atropine annulled. I have a lantern slide from a record of this effect, which Professor Dixon gave to me.



FIG. 1.—Unpublished record from an experiment in 1906 by the late W. E. Dixon. Beat of the exposed heart of a frog. At the first mark extract from inhibited dog's heart applied; at the second mark atropine.

Nobody can say now what he had in his extract, though we may be pretty sure that it was not the labile substance now known to transmit vagus effects, and that its presence had little, if any, connexion with the inhibition of the heart from which it was extracted. Probably it was choline. It is beyond doubt, however, that Dixon, following Elliott's suggestion concerning adrenaline, had at that early date a conception of the general nature of the mechanism which later evidence has completely justified.

ACTIONS OF ACETYLCHOLINE

From 1906 to 1921 there is a gap in the record of direct contributions to the theory of chemical transmission. The idea was there, in the backs of many minds, but waiting for direct evidence to stimulate its further development. Mention should be made, however, of two investigations on the action of a substance which was to play a part of central importance in these developments when they came.

As long ago as 1900 Reid Hunt had begun experiments on depressor constituents of the suprarenal gland. He could not find enough choline to account for the depressor action of an extract, and he was led, in 1901, to suggest that the excess of activity might be due to an unstable and more active derivative of choline. Since the additional activity was not abolished by atropine, it now seems more probable that Hunt was dealing with histamine, the action of which was not known till much later; but he had the idea of a choline derivative, and it led him to try the action of a number of esters, which were made for him by Taveau (Hunt and Taveau, 1906). Among these was the acetic ester, acetylcholine, which Hunt found to have an action like that of choline, but about one thousand times as strong. This observation was published in the same year, and, indeed, at the same meeting of the British Medical Association as Dixon's first tentative mention of his heart-vagus experiment.

One other happening in 1906 should be noted in passing. It was then that Howell (1906, 1908) began to put forward the evidence which led him to suggest that vagus impulses inhibit the heart by mobilizing potassium ions. This is sometimes quoted as an early forecast of our present knowledge of chemical transmission, but its interest seems to me to lie in a different direction.

Some seven or eight years later, having come across acetylcholine accidentally, as a constituent of a particular sample of ergot and therefore as a product of nature, I was led to make a detailed study of its action (Dale, 1914). This, I think, gave the first hint that acetylcholine might have an interest for physiology. It was found to be a very unstable substance, even outside the body; but when it was injected into the circulation its effects, though immediate and intense, were so extraordinarily evanescent that I suggested, rightly as it now appears, that it was probably hydrolysed with great rapidity by an esterase in the blood, being split into acetic acid and the comparatively inactive choline. Then I was struck by the remarkable fidelity with which it reproduced the various effects of parasympathetic nerves, inhibitor on some organs and augmentor on others—a

fidelity which I compared to that with which adrenaline reproduces the effects of the other, true sympathetic, division of the autonomic system. Thus we now had knowledge of two substances, both with intense activities; both, by reason of their liability to the actions of different body ferments, having similarly evanescent effects; and each reproducing, with a similar fidelity, the effects of one of the two main anatomical divisions of the autonomic nervous system. There was this difference between the two cases, however, that adrenaline was already known as a natural substance, formed in and secreted from the cells of the suprarenal medulla into the blood, and thus, by its direct action from the blood stream, supplementing the effects of sympathetic nerves which it so accurately reproduces. This natural occurrence gave an added plausibility to Elliott's suggestion that adrenaline intervened in the direct effects of sympathetic nerve impulses; whereas in 1914, as I was bound to admit, we had no evidence at all that acetylcholine was a constituent of any part of the animal body, and many years, in fact, elapsed before we found it there.

There was yet another action of acetylcholine, which seemed at the time to have no relation to any physiological function. Its parasympathetic effects, produced by extremely minute doses, were all readily annulled by a small dose of atropine. Only when these had thus been suppressed was it recognized that larger, but still small, doses of acetylcholine had a stimulating action on ganglion cells, recalling that of nicotine. This is an action shown by many bases of the quaternary ammonium type, to which acetylcholine belongs. To the nicotine-like action of acetylcholine belong also its later-described stimulating effects on voluntary muscle—on normal muscles of some lower vertebrates and motor-denervated muscles of mammals (Riesser, 1921, Frank, Nothmann, and Hirsch-Kauffmann, 1922, 1923, Dale and Gasser, 1926). We shall see later that this action also has quite recently acquired a physiological significance of very great interest. For the time, however, it was only possible to recognize the fact that acetylcholine, in common with other choline esters indeed, but with a unique intensity and evanescence, exhibited these two types of action, which I referred to as its "muscarine" and "nicotine" actions.

THE EXPERIMENT OF OTTO LOEWI ON VAGUS INHIBITION

The observations recorded above were completed in the fateful year 1914, when the outbreak of war diverted all scientific energies from their normal applications. The next chapter in our story, accordingly, opened seven years later, in 1921: but it was one of outstanding importance. In that year (1921) Otto Loewi published his simple, elegant, and convincing demonstration that the vagus nerve produces its effect on the frog's heart by liberating an inhibitor substance. He showed that this substance, as obtained in the fluid filling the heart, can be transferred to another heart, and there reproduce the vagus effect.

The experiment demanded no special technique or apparatus; it might, one reflected, have been made at any time during the fifteen years or more since the idea of a specific chemical transmission of nervous effects first took shape. It needed only that touch of scientific courage which has led to the making of some of the most important discoveries by direct and simple means.

This classical experiment formed the starting-point for a series of others, in Loewi's laboratory and elsewhere, in which the liberation of a substance having properties similar to those of the vagus substance, and similarly transmitting parasympathetic effects, has been shown to accompany the reflex production of the autonomic actions of the third cranial nerve (Engelhart, 1931), and the production by artificial stimulation of the effects of the

chorda tympani on the salivary gland and the tongue (Babkin, Alley, and Stavratsky, 1932), Gibbs and Szelöczy, 1932, Bain, 1932, Henderson and Roepke, 1933, and Feldberg, 1933).

RESEMBLANCE OF THE "TRANSMITTER" TO ACETYLCHOLINE

Loewi not only demonstrated the liberation of an inhibitor substance transmitting the effect of the vagus to the frog's heart; he was able, even with the minute traces obtained, to examine the properties of the substance in several directions; and these properties were found to correspond, in every test, to those of acetylcholine. Atropine annulled the action of the transmitter, but did not prevent its liberation by the vagus. The transmitter was rapidly destroyed by an esterase present in the heart muscle, and its activity could be restored by acetylating the residue. Of special interest, and of great value for further progress, was the discovery that eserine (physostigmine) inhibited the action of the esterase; so that the actions of atropine and eserine, in paralysing and intensifying respectively the action of the vagus on the heart, were fully explained by the new knowledge that this action was transmitted by something indistinguishable from acetylcholine. This effect of eserine was given a more general application, when Engelhart (1930) in Loewi's laboratory, and Matthes (1930) in my own, showed that, even in very high dilutions, it blocked the destructive action of a blood esterase on acetylcholine. We thus came to regard eserine as an indicator for the action of an unstable choline ester, like acetylcholine. Whenever eserine was found to intensify or prolong a nervous effect there was now reason to suspect that this was transmitted by the release of an unstable choline ester. To use a terminology which I recently suggested, eserine became an indicator of "cholinergic" effects.

CHEMICAL TRANSMISSION OF SYMPATHETIC EFFECTS

Before we pass to later developments concerning acetylcholine it will be convenient to deal with those in another chapter of the story, which also began with Loewi's observations.

You will remember that the vagus of the frog contains fibres which join it from the sympathetic chain, and that the effect of these sometimes predominates, so that stimulation of the mixed nerve may cause acceleration and augmentation of the heart beat, instead of inhibition. Loewi found that in such cases the fluid in the heart would transmit an accelerator, adrenaline-like effect to another heart; so that Elliott's speculation, as to the meaning of the similarity of sympathetic effects to those of adrenaline, received at last a direct experimental justification.

Further progress in our knowledge of this chemical transmitter of the peripheral effects of true sympathetic nerves has come largely from Cannon's laboratory at Harvard, and from the researches of visitors from other countries who have worked there. Cannon's recent researches have been largely concerned with the demonstration that, when the lower end of the sympathetic chain is stimulated in a cat deprived of its suprarenal glands, something passes into the blood which produces, at a distance, effects of sympathetic stimulation on other organs (Cannon and Bacq, 1931). The substance seems to be liberated largely in connexion with the pilomotor action. To avoid a premature suggestion as to its chemical nature, Cannon refers to this transmitter of sympathetic effects as "sympathin." There is an obvious probability in favour of its being the substance, natural to the body, and reproducing sympathetic effects with such remarkable precision—adrenaline itself, as suggested long ago by Elliott.

Bacq of Liège, for a time a co-worker with Cannon, has shown that when the cervical sympathetic nerve is stimulated sympathin appears in the aqueous humour of the eye (1933); just as Engelhart had found that, when the pupil was caused to constrict by the incidence of light, something like acetylcholine appeared in the same fluid. Bacq has been able to apply to the sympathin so obtained certain chemical and spectrographic tests, the results of which seem to make it clear that it is, at least, a catechol derivative with an aminated side-chain—in other words, that it is either adrenaline itself or a very closely related substance. There is a complication, due to recent observations of Cannon and Rosenblueth (1933), which suggest that sympathin, as it passes into the blood from the site of its liberation, may produce on distant organs only the augmentor, or only the inhibitor, effects of sympathetic nerves. It would be possible, indeed, to name substances closely related to adrenaline, but producing in the one case mainly the motor, and in the other case mainly the inhibitor, actions of adrenaline and sympathetic nerves; but we have no kind of warrant for regarding these as substances likely to occur naturally in the body. Cannon supposes that the actual transmitter is a substance capable of producing either type of effect, as adrenaline does, according to the type of receptive substance which it finds, and combines with, in the effector cell. He imagines that two types of such combination may occur, producing what he calls "sympathin E" and "sympathin I," which have augmentor and inhibitor effects respectively; and it is these combinations, he believes, which escape to some extent into the blood stream. It should be said, I think, that the behaviour of the substance transmitting parasympathetic effects, concerning which more is known, provides no analogy for this conception. Whether liberated by an augmentor or an inhibitor nervous effect, it behaves like acetylcholine itself, and produces on all cells which are sensitive to that substance its characteristic effects, whether inhibitor or augmentor.

We may safely leave the details of the chemical transmission of peripheral sympathetic effects to the further investigations of Cannon* and his school, and return to the transmission of the peripheral effects of parasympathetic nerves. We shall find there a mechanism which is beginning to have a much wider application than could have been suspected, even a few months ago.

ACETYLCHOLINE, A NATURAL BODY CONSTITUENT

We have seen that Loewi's vagus substance, and that liberated in the transmission of other parasympathetic effects, showed all the properties of acetylcholine, so far as these could be examined. There was a proper reluctance at first to assume identity with that substance, in default of any chemical evidence of its occurrence in the animal body. That impediment, it seems to me, was largely removed when Dudley and I (1929), looking for another substance in extracts from the spleen of the ox and the horse, came by accident on an activity like that of acetylcholine, and succeeded in isolating that substance in a quantity sufficient for clear chemical recognition. Since then Kapfhammer and his colleagues in Freiburg (Kapfhammer and Bischoff, 1930; Bischoff, Grab, and Kapfhammer, 1931) claim to have found it in much larger quantities in almost every organ of the body; but my laboratory has failed to confirm their observations. In only one other tissue, the human placenta (Chang and Gaddum, 1933), have we found evidence of the presence of acetylcholine in amounts of the same order as those occurring in the spleens of the large ungulates. Neither

* Professor Cannon's own account of the full evidence concerning this function, and of his present interpretation of it, will shortly be published in his recently delivered Kober Lecture, of which he has kindly allowed me to see the manuscript.

in the spleen nor in the placenta does its occurrence in these large amounts have any obvious relation to the action of parasympathetic nerves, or to any other known physiological function. We may reasonably hope that the meaning of its presence in such organs will some day be revealed; and, meanwhile, we may be satisfied with the evidence that acetylcholine is a normal product of the animal body, and that there is no reason, on that account, for suspecting that the choline ester transmitting parasympathetic effects is any other than this one.

DIFFICULTIES CAUSED BY SOME ACTIONS OF ATROPINE

A difficulty which some have found in accepting this identification is connected with the effects of atropine. There are some parasympathetic effects, such as the action of the vagus on the intestine, and the vaso-dilator actions of parasympathetic nerves in general, which are resistant to atropine, though the otherwise similar actions of injecting or applying acetylcholine are readily abolished by it. But, as Gaddum and I (1930) have argued in dealing with cases of this kind, the fact that such nerve effects are potentiated by eserine gives good reason for believing that they are transmitted by a sensitive choline ester; and, since these muscarine actions of all choline esters are equally liable to annulment by atropine, the resistance of the nervous actions to that alkaloid must have some other explanation. We certainly do not avoid the difficulty by talking of the transmitter as an "acetylcholine-like substance." If the atropine anomaly were sufficient to exclude the identification of the transmitter as acetylcholine, it could not be a choline ester at all, as the action of eserine shows it to be. Gaddum and I suggested that in such cases the nerve impulses liberate acetylcholine so close to the reactive structures that atropine cannot intervene, whereas it can prevent its access to them when it is artificially applied from without.

PHYSIOLOGICAL RECOGNITION OF ACETYLCHOLINE

To settle the matter beyond discussion we should presumably need to collect a sufficient quantity of the substance transmitting the effects of nerve impulses to enable it to be chemically identified. Perhaps some day we may be able to do this, but there is no immediate prospect of its achievement.

In default of this possibility there are various tests we can apply, the cumulative effect of which is to make the identification practically certain. It can be shown that the transmitter is a very unstable choline ester, rapidly destroyed at a definitely alkaline reaction, even at room temperature; that it is rapidly destroyed by the esterase present in blood, which the Stedmans have shown to be an enzyme specific for esters of choline, but that it is protected from this enzyme by a small proportion of eserine. We can then determine its activity in terms of acetylcholine by a number of different physiological tests—by its vaso-dilator depressor action in the rabbit or cat, its inhibitor action on the isolated frog's heart or rabbit's auricle, its stimulation of contracture in the frog's voluntary muscle, or the body wall of the leech sensitized to it by eserine. This reaction of leech muscle, recently brought to my laboratory from Germany by Dr. Feldberg, is of amazing sensitiveness, and of special value for detecting and measuring acetylcholine in blood. And when it is found that, in all these different reactions, the activity of a solution containing the parasympathetic transmitter is matched by the same strength of acetylcholine, we can be practically certain that we are dealing with that substance and with no other choline ester. And since it is the only choline ester known to occur in the body at all there seems no reason to look further. Feldberg and I (Dale and Feldberg, 1933) have been able, for example, to collect the substance released in the wall

of the dog's stomach when the vagus nerves are stimulated, and have found it to correspond exactly with acetylcholine in all these different respects. We suggest that the mode of transmission of vagus impulses is not likely to change abruptly on passing from stomach to intestine. It seems to be very much more probable that the actions of the vagus on the small intestine, and of the pelvic nerve on the large intestine, are similarly transmitted by the release of acetylcholine, but in such proximity to the reactive structures that atropine interferes but little with its action. This, however, is a point on which direct evidence has yet to be obtained.

CLASSIFICATION BY CHEMICAL FUNCTION. CHOLINERGIC AND ADRENERGIC NERVES

In general, it holds good that acetylcholine, or some choline ester indistinguishable from it, is the chemical transmitter of peripheral parasympathetic effects, while that for the actions of true sympathetic nerves is either adrenaline or some closely related substance. To this broad correspondence, however, between chemical function and anatomical origin, there are exceptions. It seemed to me that we needed words to indicate the functions of nerve fibres as regards the chemical transmission of their activity, without reference to their anatomical connexions, and I have proposed the use of the adjectives "cholinergic" and "adrenergic" in this sense (Dale, 1933).

The most obvious exceptions to the general correspondence are those of nerve fibres which arise from sympathetic ganglion cells, but are cholinergic. Langley (1901) and Elliott (1905) found it impossible to detect a stimulation by adrenaline of sweat glands in the cat's foot or the human hand, corresponding to the effect on them of sympathetic nerve impulses. The sweat glands of the cat, indeed, have long presented a puzzle and an anomaly to the systematic pharmacologist; for they respond but little to adrenaline, but they are stimulated to profuse secretion by substances like pilocarpine and acetylcholine, and their activity is readily paralysed by atropine and unaffected by ergotoxine, though their nerves belong to the true sympathetic system. The anomaly would obviously be explained if these particular sympathetic fibres were cholinergic, and not adrenergic like most.

Quite recently Feldberg and I have obtained direct evidence that this is the case.

We perfused the foot of a cat, the hairless pads of which carry the sweat glands, with Locke's solution containing a little eserine. As soon as we stimulated the sympathetic nerve supply to the foot and caused sweating on the pads, acetylcholine appeared promptly in the venous fluid. It disappeared when the glands returned to rest and reappeared on renewing the stimulation; but it did not appear at all, though the stimulation was still effective on the blood vessels of the foot, if the hairless pads, with their sweat glands, were excluded from the perfusion.

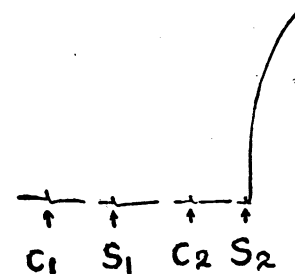


FIG. 2.—Isolated leech muscle treated with eserine, tested with effluents from perfused cat's foot. C_1 and C_2 = control fluids without stimulation. S_1 = fluid during sympathetic stimulation, from foot with sweat glands excluded from perfusion. S_2 = fluid during exactly similar stimulation, from foot with sweat glands included in perfusion.

We cannot perform an experiment of this kind on man, but the cholinergic nature of the nerve supply to the human sweat glands is clearly indicated by their failure to respond to adrenaline, their response to pilocarpine

with profuse secretion, and their paralysis by atropine. In some other animals, however, the nerve supply to the sweat glands appears to be adrenergic—for example, in the horse.

Another effect of sympathetic nerve stimulation which is not reproduced by adrenaline is the vaso-dilatation in the buccal mucosa in the dog, long ago described by Dastre and Morat (1880) as caused by stimulating the cervical sympathetic nerve in that animal. Rogowicz (1885) showed that when the voluntary muscles of the dog's cheek were denervated they responded with contracture when the cervical sympathetic nerve was stimulated. This was closely similar to the effects which we (Dale and Gaddum, 1930) elsewhere attributed to the leak on to the sensitized muscle fibres of acetylcholine, released to transmit a vaso-dilator effect; and Euler and Gaddum (1931), who reinvestigated this Rogowicz phenomenon, concluded that it was, indeed, due to the presence in the dog's cervical sympathetic nerve of fibres which were truly sympathetic, but acted by release of acetylcholine—being what we should now call cholinergic sympathetic fibres.

A study of the similar contracture of the muscles of the hind limb deprived of their motor nerves, originally described by Sherrington, led Gaddum and me (1930) to the conclusion that the antidromic vaso-dilatation, produced by impulses in side branches of sensory nerve fibres to the small arteries, was also cholinergic. More recent evidence by Hinsey and Cutting (1933) suggests that the Sherrington phenomenon is due to sympathetic cholinergic fibres joining the sciatic plexus through the grey rami, and not, as had been thought, to antidromic impulses in sensory fibres. The question, whether the axon-reflex or antidromic vaso-dilatation is cholinergic, is therefore once more open, and will require direct evidence for its decision.

NICOTINE ACTIONS OF ACETYLCHOLINE

Mention of the pseudomotor phenomena leads us to the other aspect of the action of acetylcholine—what I have termed its "nicotine" action—the physiological interest of which has only very recently begun to appear. This was a question which had puzzled me for many years. Why should Nature use, as the transmitter of parasympathetic effects to involuntary muscles and gland cells, such a substance as acetylcholine, having not only the action directly appropriate to this purpose, but, in addition, a "nicotine" action on ganglion cells and voluntary muscle which seemed entirely irrelevant to it?

The ruling conceptions of the mode of transmission of nerve impulses across synapses to ganglion cells, or from motor nerve endings to the end-plates of voluntary muscle fibres, made it difficult to speculate on any intervention of acetylcholine in such cases. Only in the past few months have the experimental facts demanded a serious consideration of such a possibility. Some years ago Witanowski (1925) detected the presence of something like acetylcholine in extracts of sympathetic ganglia, and Chang and Gaddum (1933) came across it again, using tests which gave clearer evidence of its identity. In both cases it was found also in the cell-free nerve, and the significance of the observations was not clear. Kibjakow (1933), however, published a description of experiments in which he had artificially perfused the superior cervical ganglion of a cat, and found that, when the preganglionic nerve was stimulated, something appeared in the venous fluid which acted as a stimulus to the ganglion cells on reinjection, as shown by the contraction of the nictitating membrane. He suggested that the impulses were transmitted across the synapse by the release of this substance, and Chang and Gaddum, in the light of their own observations, suggested that Kibjakow's substance might be acetylcholine.

TRANSMISSION OF NERVE IMPULSES TO THE ADRENAL MEDULLA

An encouragement to the further consideration of this possibility, and to its ultimate testing by experiment, was furnished by Feldberg and Minz's (1933) discovery that, when the splanchnic nerve supply to the suprarenal medulla is stimulated, acetylcholine appears in the blood of the suprarenal vein, if its destruction is prevented by eserine; so that acetylcholine here transmits, to the medullary cells, the nerve impulses which cause them to secrete adrenaline into the blood. Now the suprarenal medullary cells are morphologically equivalent to sympathetic ganglion cells, and at least some sympathetic preganglionic fibres appear to end in direct relationship to them. Further experiments, which Feldberg has now completed in my laboratory, have shown that, in harmony with this conception, it is chiefly the nicotine action of acetylcholine which is concerned in its action on these medullary cells, and in the transmission to them of the effects of splanchnic impulses.

TRANSMISSION OF IMPULSES AT GANGLIONIC SYNAPSES

With this analogy before them, Feldberg and Gaddum (1933) have proceeded to a direct test of the possibility that the transmission of a nerve impulse across the synapse in a ganglion is effected by the release of acetylcholine. They have used Kibjakow's technique for perfusing the superior cervical ganglion, recording contractions of the nictitating membrane as an index of the activity of the ganglion cells. They obtained no result when plain Locke's solution was used, but when a very low concentration of eserine was added to it an active substance appeared in the venous fluid whenever the preganglionic nerve was stimulated, but only then. And this substance, by all the tests with which we are now familiar, corresponds so exactly in its properties and actions to acetylcholine that there is no reason to doubt its identity.

The conception of the transmission of a nervous impulse across a synapse by the release of such a substance, and by the action of this substance as the direct stimulant of the ganglion cell, though it satisfies my own desire to bring the nicotine action of acetylcholine into the physiological picture, is not without its difficulties, and it will have to justify itself to win general acceptance. One thing is clear—namely, that when preganglionic impulses arrive in the ganglion acetylcholine is there released in such an amount that it not only may but *must* stimulate the ganglion cells to their only known form of activity, in the output of impulses in the post-ganglionic fibres, corresponding to those which arrive in the preganglionic fibres.

Several recent investigations (Bishop and Heinbecker, 1932, Brown, 1934, and Eccles, 1934) have shown that a single impulse in a preganglionic fibre produces a corresponding single impulse in post-ganglionic fibres, and that the delay at the synapse is very short. One can only suppose that each impulse must cause the release, in immediate proximity to the ganglion cell, of a minute charge of acetylcholine, which fires off a post-ganglionic impulse and then immediately disappears. At this early stage we must wait for the additional evidence, which is almost daily accumulating in my laboratory; and I can only say that it seems to be wholly in favour of some such conception.

TRANSMISSION OF NERVOUS EXCITATION TO VOLUNTARY MUSCLE

Finally we are led, by the analogy which I mentioned at the beginning of my lecture, to inquire whether acetylcholine may not intervene, also by virtue of its nicotine

action, in the transmission of the effect of a motor nerve impulse to a voluntary muscle fibre. For a good many years there have been indirect indications of such a possibility. If we extend our view to a wider range of animal types, the demarcation between the functions and the modes of innervation of striated muscle on the one hand, and of plain muscle on the other, is not everywhere so sharp as we are apt to regard it in dealing with the mammal. The intestine of the tench, a common fresh-

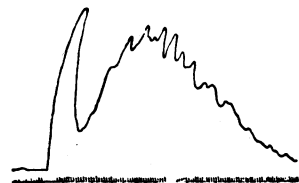


FIG. 3.—Isolated intestine of *Tinca vulgaris*. Acetylcholine (1 in 50,000) added at mark, producing quick contraction of striated muscle coat and slow contraction, with rhythm, of unstriated coat. (Reproduced from Mehes and Wolsky, *Arb. d. Ungar. Biol. Forsch.*, vol. v, p. 150.)

water fish, has two muscle coats—one striated, the other plain—and both are innervated by fibres from the vagus. Acetylcholine stimulates the former to a relatively rapid and short contraction, the latter to a prolonged increase of tone and rhythm; and its effect on the striated coat is of the nicotine type, being paralysed by curare, while that on the unstriated coat is of the muscarine type, being paralysed by atropine (Mehes and Wolsky, 1932).

The dual activities of acetylcholine, and the corresponding paralytic effects on them of atropine and curare, are similarly plain when we compare its actions on the unstriated sphincter of the mammalian pupil, and on that of the bird, which consists of striated fibres. The fact that acetylcholine stimulates certain normal voluntary skeletal muscles in lower vertebrates, and those of mammals after degeneration of their motor nerves, I have already mentioned.

There has been evidence, from several observers, of the appearance of something like acetylcholine in the venous effluent from a perfused voluntary muscle when its nerve is stimulated (Hess, 1923, Shimidzu, 1926, Brinkman, 1924, 1925, and Plattner, 1932). In all these cases, however, the nerve stimulated was a mixed nerve, containing sensory and sympathetic fibres as well as voluntary motor fibres, and the evidence did not clearly suggest, even to its authors, that the appearance of acetylcholine was connected with the transmission of voluntary motor impulses. The idea involved the same kind of difficulties as that of its action as transmitter at ganglionic synapses. With the direct evidence now before us with regard to transmission in a ganglion, it seemed that an effort must be made to get a clearer test of the possibility of its acting similarly in the case of motor nerve and voluntary muscle.

The few experiments which Feldberg and I have as yet completed have given results

which seem to be so definitely favourable to such a conception as to justify reference to them even at this early stage.

The hypoglossal nerve carries purely motor fibres to the voluntary muscle of the tongue, mixed only with sympathetic fibres, which can be caused to degenerate by removing the superior cervical ganglion. If the tongue of a cat is perfused with Locke's solution containing a small amount of eserine, and is made to contract by stimulating this purely motor nerve supply, acetylcholine appears in the outflowing solution while the stimulation is continued, disappears during a follow-

ing period of rest, and reappears when effective stimulation is resumed.*

The observations are too new and too incomplete for detailed analysis of their meaning. We must bear in mind the fact that the responses of skeletal muscles, whether normal or denervated, to the artificial application of acetylcholine, either to the surface of the isolated muscle or through the circulation, take the form of relatively slow and weak contractures, and not of twitches. There is, indeed, no reason to expect that, when reaching successive muscle fibres by diffusion from the surface or from the blood vessels, acetylcholine would produce effects of the same type as those which might result from its sudden and simultaneous release in immediate proximity to the end-plates of all the fibres of the muscle, and its equally sudden removal, after the manner of the "excitatory substance" admitted by Adrian as a possibility. It might be suggested, however, that acetylcholine, even if thus liberated by motor nerve impulses, would probably be concerned with contractures rather than with normal twitches, and perhaps with the function of special, slow-contracting fibres. It must be admitted that these are still possibilities; but the apparent function of

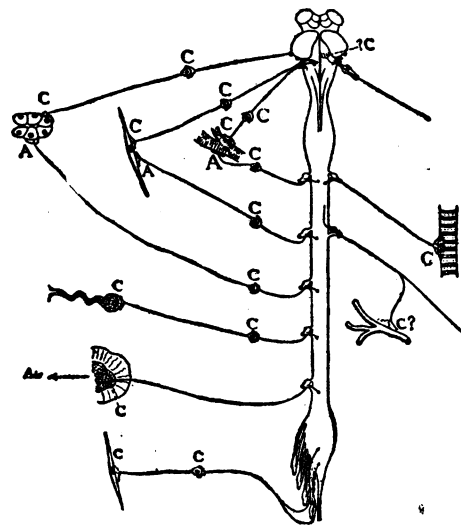


FIG. 5.—Diagram of peripheral nervous system. At points marked C there is evidence of a cholinergic transmission, at those marked A of an adrenergic transmission. Doubtful cases marked ?.

acetylcholine in transmitting the effects of impulses to nerve cells encourages one to expect a more general function for it in the transmission of motor nerve impulses to voluntary muscle. In any case, the evidence, even so far as it has been obtained, seems to give promise that we may soon be able to complete the picture of the transfer of excitation, at all cytoneural junctions in connexion with the peripheral nervous system, by the liberation of chemical transmitters.

One further question is almost inevitable. Is this conception to be limited to the peripheral nervous system, or are we to expect its extension to the synapses of the central grey matter itself? With no direct experience of central nervous physiology, I cannot properly allow myself merely to speculate. Sherrington, you will recall, with his unique authority, has envisaged the general possibility of a chemical mechanism at central synapses. And it should be mentioned that Dikshit (1934), an Indian pharmacologist working in Professor A. J. Clark's laboratory in Edinburgh, has very recently shown that minute quantities of acetylcholine, if injected into the fluid of the cerebral ventricles, reproduce with a striking fidelity the

* An entirely similar result has, even more recently, been obtained with the muscles of the leg, excited to contraction by stimulating the ventral spinal roots, after extirpation of the lumbar sympathetic chain.

effects of central stimulation of the vagus on respiratory activity; and he suggests liberation of this substance in the centres by sensory impulses in the vagus.

CONCLUSION

I can best summarize the account which I have given, of research on the chemical mechanisms for the transmission of nervous stimuli and of its most recent extensions, by putting before you a rough diagram of peripheral synapses and endings of efferent nerves. In each case, where the evidence seems fairly clear, I have indicated a cholinergic mechanism of transmission by the letter C, an adrenergic mechanism by the letter A. (Fig. 5.)

You will see at once that the C's greatly preponderate, just as the later section of my lecture has dealt exclusively with acetylcholine. We get an impression of the cholinergic mechanism as having the more general application in the functions of the nervous system, and probably an earlier origin in evolution, and of the adrenergic mechanism as a more specialized and probably a more recent development. I believe that such a conception would have been congenial to the thought of one of the greatest of British physiologists, and one of the founders of the Cambridge school, the late W. H. Gaskell. You will see that in some places the diagram has notes of interrogation, representing points at which further evidence is required to justify a definite statement. There might have been many more, for the whole field of inquiry is full of unknown details, asking for investigation, and attractive to scientific curiosity. I have tried to show you the main outlines of the map, as they are now beginning to emerge.

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DISEASE AT ITS ONSET

WITH SPECIAL REFERENCE TO OCULAR
MANIFESTATIONS

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Biophysics and biochemistry have taught us that the living body is an admirably equipped laboratory in which Nature is continually performing experiments.¹ These experiments resemble those conducted in every physiological laboratory in so far that some succeed while others fail. When Nature's experiments are successful we are not conscious that anything is happening: everything goes like clockwork, and we feel in good health. When, on the other hand, the experiments fail we become aware of unpleasant sensations, which we have learned to recognize as symptoms of disease. Health, therefore, must be regarded as a dynamic rather than as a passive state. We keep healthy as a result of a perpetual struggle to maintain physiological equilibrium in the reactions constantly taking place between the cells of the body and their nutrient capillaries. Physico-chemical changes, therefore, which originate in the capillary system are, in all probability, the first departure from health, and the cause of the earliest symptoms of disease.

The part played by the capillaries is quite distinct from that played by the heart and other blood vessels. All metabolic changes take place through their walls; consequently they form the most active, purposive, and dynamic part of the vascular system. Their contractility is controlled not only by vasomotor nerves but also by chemical stimuli, the former acting as a coarse and the latter as a fine adjustment. Krogh² has demonstrated that the capillaries are not simply tubes through which blood flows. They do not respond passively to the amplitude of the pulse wave in the arteries. They are really a constituent part of the tissues in which they lie, and their blood circulation is regulated and controlled by the requirements of the individual cells of the structures they supply. All the important business of life is transacted through the walls of the capillaries.

Bryson³ has suggested that in every part of the body the functioning unit and its associated capillaries constitute an organ in miniature. That is a simple, but it is also a most useful, conception. It implies that the health of every organ depends upon the quality and the quantity of the blood circulating in its capillary system. It is to be remembered, however, that in normal circumstances all the capillaries of an organ are not in action at the same time. They work in shifts: while some are open and active, others are closed down and idle. Herring⁴ calls this "the law of fluctuation," and points out that in all living structures the alternation of periods of activity with periods of rest is essential to health.

CIRCULATION IN THE EYE

In the eye, with the help of the ophthalmoscope, a complete circulation—arteries, veins, and capillaries—is exposed to view, and can be studied as an integrated whole, with a completeness quite impossible in any other part of the living body. Thanks to the transparency of the normal retina we can see the blood in the central artery and can follow it into the finest arterioles and, if we make use of Friedenwald's⁵ yellow-green light, we can trace the capillary network in wonderful detail.

From the clinical standpoint the retina, although it is such a very complicated structure, can be conveniently